

IN THE CLAIMS

The claims are as follows.

1. (Previously Presented) A vector for directional cloning comprising a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector, once digested with the first and second restriction enzymes yields a vector backbone that when ligated to a DNA fragment comprising an open reading frame flanked by an end generated by *SgfI* and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, yields a recombinant vector comprising the open reading frame, wherein the site in the recombinant vector formed by ligation of the 3' TA overhang and the end generated by *SgfI* is 5' to the open reading frame, and wherein if the vector backbone has an open reading frame that is 5' to the site and is in frame with the open reading frame 3' to the site, the vector backbone includes a promoter that is operably linked to the open reading frame which is 5' to the site.
2. (Original) The vector of claim 1 wherein the second and third restriction enzymes are the same.
3. (Original) The vector of claim 1 wherein the second and third restriction enzymes are different.
4. (Original) The vector of claim 1 wherein the second restriction enzyme is *PmeI*, *EcoRV* or *BalI*.
5. (Previously Presented) The vector of claim 1 wherein the second restriction enzyme is *PmeI*, *DraI*, *EsaBC3I*, *HincII*, *HpaI*, *SciI* or *SwaI*.
6. (Previously Presented) The vector of claim 1 wherein the second restriction enzyme is *AluI*, *BalI*, *BfrBI*, *BsaAI*, *BsaBI*, *BsrBI*, *BtrI*, *Cac8I*, *CdiI*, *CviJI*, *CviRI*, *Eco47III*, *Eco78I*,

EcoICRI, EcoRV, FnuDII, FspAI, HaeI, HaeIII, Hpy8I, LpnI, MlyI, MsI, MstI, NaeI, NruI, NspBII, OliI, PmaCI, PmeI, PshAI, PsiI, PvuII, RsaI, ScaI, SmaI, SnaBI, SrfI, SspI, SspD5I, StuI, XcaI, XmnI, or ZraI.

7. (Original) The vector of claim 1 wherein the restriction enzyme that generates a 3' TA overhang is *SgfI*.

8. (Original) The vector of claim 1 which further comprises an open reading frame which includes the recognition site for the first restriction enzyme.

9. (Original) The vector of claim 1 which comprises an appropriately positioned ribosome binding site 5' to the nucleotide cleaved by the first restriction enzyme.

10. (Previously Presented) The vector of claim 1 wherein ligation generates the following sequence in the recombinant vector AAGGAGCGATCGCYATG (SEQ ID NO:69) or X₁X₂X₃GCGATGCCATG (SEQ ID NO:70), wherein X₁-X₃, X₂X₃G or X₃GC is a codon which is not a stop codon, and wherein Y is A, T, G or C.

11. (Original) The vector of claim 1 wherein ligation generates the following sequence in the recombinant vector X₁X₂X₃GTTTY₁Y₂, wherein X₁X₂X₃ is a codon in an open reading frame which is not a stop codon and Y₁ and Y₂ each =A, Y₁ = A and Y₂ = G or Y₁ = G and Y₂ = A.

12. (Original) The vector of claim 1 wherein ligation generates the following sequence in the recombinant vector X₁X₂X₃GTTTY₁Y₂, wherein X₁X₂X₃, X₂X₃G or X₃GT is a codon in an open reading frame which is not a stop codon and Y₁ is not A when Y₂ is A or G, or Y₁ is not G when Y₂ is A.

13. (Withdrawn) A vector comprising a first open reading frame which includes a recognition site for a first restriction enzyme that generates a 3' TA overhang and a recognition site for a second restriction enzyme that is not in the open reading frame generates blunt ends, which

vector, once digested with the first and second restriction enzymes and ligated to a DNA fragment comprising a second open reading flanked by an end generated by *SgfI* and a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, yields a recombinant vector comprising a third open reading frame comprising the first and second open reading frames, which third open reading frame encodes a fusion peptide or protein.

14. (Withdrawn) A vector comprising a ribosome binding site which optionally overlaps by one nucleotide with a *SgfI* recognition site and a recognition site for a first restriction enzyme that generates blunt ends, which vector, once digested with *SgfI* and the first restriction enzyme and ligated to a DNA fragment comprising an open reading frame encoding a peptide or polypeptide flanked by

5' CGCCATGX₁Y₁ (SEQ ID NO:2)
3' TAGCGGTACX₂Y₂ (SEQ ID NO:71)

and a blunt end generated by a second restriction enzyme that has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, yields a recombinant vector which encodes the peptide or polypeptide, wherein X₁ is the first codon which is 3' to the start codon for the open reading frame, wherein X₂ is the complement of X₁, wherein Y₁ is the remainder of the open reading frame, and wherein Y₂ is the complement of Y₁.

15. (Withdrawn) A support comprising a plurality of recombinant vectors, two or more of which comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a first restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the first open reading frame flanked by an end generated by *SgfI* and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

16. (Withdrawn) The support of claim 15 wherein the vector further comprises a second open reading frame 3' to the promoter which second open reading frame includes the recognition site for the first restriction enzyme, which second open reading frame, when ligated to the first open reading frame, forms a third open reading frame which encodes a fusion peptide or protein.

17. (Withdrawn) The support of claim 15 wherein ligation generates the following sequence in the recombinant vector AAGGAGCGATCGCYATG (SEQ ID NO:69) or X₁X₂X₃GCGATCGCCATG (SEQ ID NO:70), wherein X₁-X₃, X₂X₃G or X₃GC is a codon which is not a stop codon, and wherein Y is A, T, G or C.

18. (Withdrawn) The support of claim 15 wherein ligation generates the following sequence in the recombinant vector X₁X₂X₃GTYY₁Y₂, wherein X₁X₂X₃ is a codon in an open reading frame which is not a stop codon and Y₁ and Y₂ each =A, Y₁ = A and Y₂ = G or Y₁ = G and Y₂ = A.

19. (Withdrawn) The support of claim 15 wherein ligation generates the following sequence in the recombinant vector X₁X₂X₃GTYY₁Y₂, wherein X₁X₂X₃, X₂X₃G or X₃GT is a codon in an open reading frame which is not a stop codon and Y₁ is not A when Y₂ is A or G, or Y₁ is not G when Y₂ is A.

20. (Withdrawn) A support comprising a plurality of recombinant vectors, two or more of which comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame comprising a second open reading frame and one or more codons which are in-frame with the second open reading frame, wherein the second open reading frame is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the second open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which DNA sequence is digested with *PmeI* and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more in-frame codons and the promoter which is 5' to an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme.

21. (Withdrawn) The support of claim 20 wherein the exchange site formed by blunt end ligation includes $N_1N_2N_3GTTTN_4N_5$, wherein $N_1N_2N_3GTTT$ is a sequence from the 3' end of the DNA sequence, wherein if $N_1N_2N_3$ do not code for a stop codon, N_4 and $N_5 = A$, or $N_4 = A$ and $N_5 = G$ or $N_4 = G$ and $N_5 = A$, or wherein $N_1N_2N_3$ code for a stop codon.

22. (Withdrawn) A support comprising a plurality of recombinant vectors, two or more of which comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and an open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxterministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

23. (Withdrawn) The support of any one of claims 15 or 20 to 22 which is multi-well plate.

24. (Withdrawn) The support of any one of claims 15 or 20 to 22 wherein the plurality of recombinant vectors each encode a different polypeptide from the same organism.

25. (Withdrawn) The support of any one of claims 15 or 20 to 22 wherein the plurality of recombinant vectors encode orthologous polypeptides.

26. (Withdrawn) The support of any one of claims 15 or 20 to 22 wherein the plurality of recombinant vectors encode paralogous polypeptides.

27. (Withdrawn) A method to prepare a support comprising a plurality of recombinant vectors or recombinant cells, comprising:

a) selecting a plurality of recombinant vectors or recombinant cells comprising recombinant vectors, wherein two or more of the recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang, which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the first open reading frame flanked by an end generated by *Sgfl* and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends; and

b) introducing the selected recombinant vectors or recombinant cells to one or more receptacles of the support.

28. (Withdrawn) A method to prepare a support comprising a plurality of recombinant vectors or recombinant cells, comprising:

a) selecting a plurality of recombinant vectors or recombinant cells comprising recombinant vectors, wherein two or more of the recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame comprising a second open reading frame and one or more codons which are in-frame with the second open reading frame, wherein the second open reading frame is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the second open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which DNA sequence is digested with *PmeI* and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more codons and the promoter which is 5' to an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme; and

b) introducing the selected recombinant vectors or recombinant cells to one or more receptacles of the support.

29. (Withdrawn) A method to prepare a support comprising a plurality of recombinant vectors or recombinant cells, comprising:

a) selecting a plurality of recombinant vectors or recombinant cells comprising recombinant vectors, wherein two or more of the recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and an open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxotministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first

DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs; and

b) introducing the selected recombinant vectors or recombinant cells to one or more receptacles of the support.

30. (Withdrawn) The method of any one of claims 27 to 29 wherein each of the selected recombinant vectors encodes a different paralogous protein.

31. (Withdrawn) The method of any one of claims 27 to 29 wherein each of the selected recombinant vectors encodes a different protein in a catabolic pathway.

32. (Withdrawn) The method of any one of claims 27 to 29 wherein each of the selected recombinant vectors encodes a different protein in a biosynthetic pathway.

33. (Withdrawn) The method of any one of claims 27 to 29 wherein each of the selected recombinant vectors encodes a different protease.

34. (Withdrawn) The method of any one of claims 27 to 29 wherein each of the selected recombinant vectors encodes a protein from the same organism.

35. (Withdrawn) The method of any one of claims 27 to 29 wherein each of the selected recombinant vectors encodes orthologous proteins.

36. (Withdrawn) A method to prepare a plurality of mutagenized recombinant vectors, comprising:

- a) providing DNAs comprising a plurality of mutagenized open reading frames flanked by a *SgfI* recognition site and a site for a first restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends; and
- b) digesting the DNAs with *SgfI* and the first restriction enzyme and ligating the digested DNAs to a vector comprising a promoter which is 5' to a recognition site for a second restriction enzyme that generates 3' TA overhangs which is 5' to a recognition site for a third restriction enzyme which generates blunt ends, which vector is digested with the second and third restriction enzymes, to yield a plurality of mutagenized recombinant vectors.

37. (Withdrawn) A method to prepare a plurality of mutagenized recombinant vectors, comprising:

- a) providing DNAs comprising a plurality of mutagenized open reading frames flanked by a recognition site for a first restriction enzyme that generates a 3' TA overhang and site for a second restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends; and
- b) digesting the DNAs with the first and second restriction enzymes and ligating the digested DNAs to a vector comprising a promoter which is 5' to a *SgfI* recognition site which is 5' to a recognition site for a third restriction enzyme which generates blunt ends, which vector is digested with *SgfI* and the third restriction enzyme, to yield a plurality of mutagenized recombinant vectors.

38. (Withdrawn) The method of claim 37 wherein the first restriction enzyme is *PmeI*.

39. (Withdrawn) A method to prepare a plurality of mutagenized recombinant vectors, comprising:

a) providing DNAs comprising a plurality of mutagenized open reading frames flanked by two restriction enzyme sites for a first restriction enzyme which is a hapaxterministic restriction enzyme and generates a first pair of non-self complementary single-strand DNA overhangs; and

b) digesting the DNAs with the first restriction enzyme and ligating the digested DNAs to a vector comprising a promoter and non-essential DNA sequences flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxterministic restriction enzyme, which vector is digested with the second restriction enzyme generating a DNA fragment which lacks non-essential DNA sequences but comprises a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs, to yield a plurality of mutagenized recombinant vectors.

40. (Withdrawn) A support comprising a plurality of mutagenized recombinant vectors prepared by the method of any one of claims 36 to 39.

41. (Withdrawn) A library of recombinant cells comprising recombinant vectors, two or more of which recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the first open reading frame flanked by an end generated by *SgI* and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

42. (Withdrawn) A library of recombinant vectors, two or more of which recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates 3' TA overhang which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the first open reading frame flanked by an end generated by *SgI* and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

43. (Withdrawn) A library of recombinant cells comprising recombinant vectors, two or more of which recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame comprising a second open reading frame and one or more codons which are in-frame with the second open reading frame, wherein the second open reading frame is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the second open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which DNA is digested with *PmeI* and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more in-frame codons and the promoter which is 5' to an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme.

44. (Withdrawn) A library of recombinant vectors, two or more of which recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and a first open reading frame comprising a second

open reading frame and one or more codons which are in-frame with the second open reading frame, wherein the second open reading frame is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the second open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which DNA is digested with *PmeI* and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more codons and the promoter which is 5' to an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme.

45. (Withdrawn) A library of recombinant cells comprising recombinant vectors, two or more of which recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and an open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxterministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

46. (Withdrawn) A library of recombinant vectors, two or more of which recombinant vectors comprise an open reading frame for a different polypeptide, wherein at least one recombinant vector comprises a promoter and an open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxterministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

47. (Withdrawn) A library of recombinant cells comprising recombinant vectors, a plurality of which comprise mutagenized recombinant vectors comprising mutagenized open reading frames of a selected open reading frame, wherein at least one mutagenized recombinant vector comprises a promoter and a mutagenized open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the mutagenized open reading frame flanked by an end generated by *Sgf*I and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

48. (Withdrawn) A library of recombinant vectors, a plurality of which recombinant vectors comprise mutagenized recombinant vectors comprising a mutagenized open reading frames of a selected open reading frame, wherein at least one mutagenized recombinant vector comprises a promoter and a mutagenized open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a vector comprising the promoter which is 5' to a recognition site for a first restriction enzyme that generates a 3' TA overhang which is 5' to a recognition site for a second restriction enzyme which generates blunt ends, which vector is digested with the first and second restriction enzymes, and

a DNA sequence comprising the mutagenized open reading frame flanked by an end generated by *SgfI* and an end generated by a third restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.

49. (Withdrawn) A library of recombinant vectors, a plurality of which recombinant vectors comprise mutagenized recombinant vectors comprising mutagenized open reading frames of a selected open reading frame, wherein at least one recombinant vector comprises a promoter and an open reading frame comprising a mutagenized open reading frame and one or more codons which are in-frame with the mutagenized open reading frame, wherein the mutagenized open reading frame is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the mutagenized open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which DNA sequence is digested with *PmeI* and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more in-frame codons and the promoter which is 5' to an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme.

50. (Withdrawn) A library of recombinant cells comprising recombinant vectors, a plurality of which recombinant vectors comprise mutagenized recombinant vectors comprising mutagenized open reading frames of a selected open reading frame, wherein at least one recombinant vector comprises a promoter and an open reading frame comprising a mutagenized open reading frame and one or more codons which are in-frame with the mutagenized open reading frame, wherein the mutagenized open reading frame is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the mutagenized open reading frame which includes a *PmeI* recognition site and is flanked at the 5' end by a recognition site for a first restriction enzyme that generates complementary single-strand DNA overhangs, which DNA sequence is digested with *PmeI* and the first restriction enzyme, and

a vector comprising a blunt end at the 5' end which is 5' to the one or more in-frame codons and the promoter which is 5' to an end generated by a second restriction enzyme which generates single-strand DNA overhangs which are complementary to the single-strand DNA overhangs generated by the first restriction enzyme.

51. (Withdrawn) A library of recombinant cells comprising recombinant vectors, a plurality of which recombinant vectors comprise mutagenized recombinant vectors comprising open reading frames of a selected open reading frame, wherein at least one recombinant vector comprises a promoter operably linked to the mutagenized open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the mutagenized open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxterministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a

second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

52. (Withdrawn) A library of recombinant vectors, a plurality of which recombinant vectors comprise mutagenized recombinant vectors comprising mutagenized open reading frames of a selected open reading frame, wherein at least one recombinant vector comprises a promoter and the mutagenized open reading frame which is flanked by two exchange sites, wherein the exchange sites are formed by ligation of

a DNA sequence comprising the mutagenized open reading frame which is flanked by at least two restriction enzyme sites for a first restriction enzyme which is a hapaxterministic restriction enzyme, which DNA sequence is digested with the first restriction enzyme to generate a first DNA fragment flanked by a first pair of non-self complementary single-strand DNA overhangs, and

a vector comprising the promoter and non-essential DNA sequences that are flanked by two restriction enzyme sites for a second restriction enzyme which is a hapaxterministic restriction enzyme, which vector is digested with the second restriction enzyme to generate a second DNA fragment which lacks non-essential DNA sequences and is flanked by a second pair of non-self complementary single-strand DNA overhangs, wherein each of the second pair of the non-self-complementary DNA overhangs is complementary to only one of the single-strand DNA overhangs of the first pair of non-self complementary single-strand DNA overhangs.

53. (Withdrawn) A method to introduce at least two recognition sites for at least two different restriction enzymes to the ends of an open reading frame, comprising:

- a) providing one or more nucleic acid sequences each comprising an open reading frame; and
- b) amplifying each nucleic acid sequence with at least a pair of oligonucleotides to yield amplified nucleic acid comprising sequences in the pair of oligonucleotides, wherein the pair of oligonucleotides has sequences which anneal to sequences in the one or more open reading

frames, wherein the sequences in the amplified nucleic acid corresponding to sequences in one of the pair of oligonucleotides comprise a restriction enzyme site for *Sg*I which is 5' to the sequences which anneal to the open reading frame, wherein the sequences in the amplified nucleic acid corresponding to sequences in the other of the pair comprises a restriction enzyme site for a first restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends, which first restriction enzyme site is 3' to the sequences which anneal to the open reading frame, and wherein the sequences in the amplified nucleic acid corresponding to sequences in the oligonucleotides are capable of being digested with *Sg*I and the first restriction enzyme.

54. (Withdrawn) The method of claim 53 further comprising adding an adenine to the 3' ends of the amplified nucleic acid to yield a modified amplified nucleic acid fragment.

55. (Withdrawn) The method of claim 54 further comprising ligating the modified amplified nucleic acid fragment to a DNA fragment having a 5' T overhang to yield a recombinant vector.

56. (Withdrawn) The method of claim 53 wherein the pair of oligonucleotides further comprise a topoisomerase I binding site at the 5' end of the oligonucleotide.

57. (Withdrawn) The method of claim 56 further comprising ligating the amplified nucleic acid fragment to a DNA fragment having blunt ends in the presence of topoisomerase I, to yield a recombinant vector.

58. (Withdrawn) The method of claim 53 further comprising digesting the amplified nucleic acid with *Sg*I and the first restriction enzyme and ligating the digested amplified nucleic acid to a DNA fragment having a blunt end and an end which is capable of ligation to an end generated by *Sg*I, to yield a recombinant vector.

59. (Withdrawn) The method of claim 58 wherein the amplified nucleic acid is purified prior to digestion.

60. (Withdrawn) The method of claim 58 wherein the amplified nucleic acid is purified after digestion and prior to ligation.
61. (Withdrawn) The method of claim 53 wherein the nucleic acid sequence is cDNA.
62. (Withdrawn) The method of claim 53 wherein the nucleic acid sequence is RNA.
63. (Withdrawn) The method of claim 53 wherein the one oligonucleotide of the pair which comprises the *Sg*I site includes an ATG 3' to the *Sg*I site which is in-frame with the open reading frame.
64. (Withdrawn) The method of claim 53 wherein two or more different nucleic acid sequences are amplified.
65. (Withdrawn) The method of claim 55, 57 or 58 further comprising transforming cells with the recombinant vector to yield recombinant cells.
66. (Withdrawn) Recombinant cells prepared by the method of claim 65.
67. (Withdrawn) The library of any one of claims 41 to 42 and 47 to 48 wherein the at least one recombinant vector comprises a further open reading frame flanked by two exchange sites, wherein the exchange sites are formed by ligation of
the recombinant vector which comprises a recognition site for a fourth and a fifth restriction enzyme site 3' to the recognition site for the restriction enzyme which generate blunt ends, wherein the fourth restriction enzyme generates a 3' TA overhang and is different than the first restriction enzyme, and wherein the fifth restriction enzyme generates blunt ends, which vector is digested with the fourth and fifth restriction enzymes, and

RESPONSE UNDER 37 CFR § 1.116 – EXPEDITED PROCEDURE

Serial Number: 10/702,228

Filing Date: November 5, 2003

Title: VECTORS FOR DIRECTIONAL CLONING

Page 20

Dkt: 341.030US1

a DNA sequence comprising the further open reading frame flanked by an end generated by *Sgf*I and a sixth restriction enzyme which has infrequent restriction sites in cDNAs or open reading frames from at least one species and generates blunt ends.